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# Source of Copper May Have Regressive Effects on Serum Cholesterol and Urea Nitrogen Among Male Fattening Lambs

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**Abstract** An experiment was conducted to determine the effect of dietary copper (Cu) on mineral profile, hematological parameters, and lipid metabolism in lambs. Eighteen Zandi male lambs (approximately 3 months of age; 17.53±1.6 kg of body weight) were housed in individual pens and were assigned randomly to one of three treatments. Treatments consisted of (1) control (no supplemental Cu), (2) 10 mg Cu/kg dry matter (DM) from copper sulfate (CuS), and (3) 10 mg Cu/kg DM from Cu proteinate (CuP). The Cu concentration was 8.2 mg/kg DM in the basal diet. Blood was sampled from the jugular vein at the beginning of the study (enrollment, before feeding Cu supplement) and at days 25, 50, and 70 of experiment. The amounts of total serum glucose, urea nitrogen, calcium, phosphorus, iron, copper, zinc, and lipids and hematological parameters were measured. Average daily gain and feed efficiency were improved ($P<0.05$) with Cu supplementation and were better for the lambs fed diet supplemented with CuP. The concentrations of serum Ca, P, and Zn were not affected by source of Cu in the diet. However, Fe concentration was lower ($P<0.01$) in the Cu-supplemented groups. Experimental treatment had no significant effects on the hematological parameters. The serum glucose concentration was not affected by treatments. However, the urea nitrogen concentrations were significantly affected ($P<0.05$) by added Cu and was lower for CuP group as compared to the lambs in the CuS and control groups. Addition of Cu had no influence ($P>0.05$) on the serum triglyceride concentration, but lambs fed with CuP supplement had lower ($P<0.05$) serum cholesterol than the CuS and control animals. These results indicated that CuP supplemented at 10 mg/kg DM improved gain and enhanced the efficiency of nitrogen in male lambs.

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## Introduction

Copper (Cu) is an essential element required by lambs and other animals for a number of biochemical functions [1]. Cu forms part of a number of enzymes called cu proenzymes. Also, dietary Cu has been shown to alter lipid metabolism in rats [2, 3], and feeding 250 mg Cu/kg DM decreases plasma and breast muscle cholesterol concentrations in poultry [4]. Limited research suggests that dietary Cu may affect lipid metabolism in ruminants, but the results are inconsistent. Engle et al. [5] reported that Cu supplementation at 20 or 40 mg Cu/kg of DM to a high-concentrate diet decreased fat depth and serum cholesterol. Engle and Spears [6] demonstrated that feeding as little as 10 or 20 mg of Cu/kg of DM with a diet containing 4.9 mg of Cu/kg of DM decreased total serum cholesterol and fat depth in Angus steers; however, 10 or 40 mg of Cu/kg of DM given to Simmental steers had no effect on performance, carcass characteristics, and lipid or cholesterol metabolism [7].

During the past several years, chelated minerals have become popular for use in mineral supplements. Ward et al. [8] reported that Cu proteinate maintained blood plasma Cu more readily in cattle and seemed to have a greater bioavailability than CuSO4. With evidence that organic minerals are more bioavailable and absorbed more readily than inorganic sources, it is not known whether lipid metabolism would be changed when fed organic complexes of Cu.

Also, sheep are more vulnerable to the effects of copper toxicity than are other species of food animal because of their less efficient excretory mechanism. Therefore, the objective of this study was to investigate the effect of different sources of

Cu on performance, blood mineral and hematological parameters, urea nitrogen, and lipid profile of growing lambs.

## Material and Methods

Eighteen Zandi male lambs (initial body weight (BW), 17.5±1.6 kg) were used as experimental animals. Animal care and use was approved by the local animal care committee. These animals were divided into three groups of six animals in each in a completely randomized design on the basis of their body weight. All the experimental animals were housed in a well-ventilated animal shed with cemented floor and provision of individual feeding and watering. While animals in the control group were fed a standard total mixed ration (TMR) containing 63/100 kg of concentrate mixture (CM), 22/100 kg of alfalfa hay, and 15/100 kg wheat straw (containing 8.2 mg Cu/kg DM), animals in the experimental groups were additionally supplemented with 10 mg Cu/kg of diet either through $CuSO_4$ (CuS) or Cu proteinate (CuP). CM contained 720 g/kg ground barley grain, 160 g/kg soybean meal, 110 g/kg wheat bran, 6 g/kg mineral mixture (without Cu), and 4 g/kg common salt. Lambs were fed the experimental diets in two daily meals (0800 and 1600 h) for 70 days. The amount of Cu supplemented was adjusted daily based on dry matter intake (DMI) of individual lambs. Lambs were weighed at the beginning of the study and then weighed in 2-week intervals.

Blood samples were collected from all the lambs at the start and days 25, 50, and 70 of experiment from the jugular vein and put into two tubes; one for measuring hematological parameters and the other for other metabolites.

Two-and-a-half milliliters of blood anticoagulated with disodium-EDTA were used for cell blood count (CBC). All tubes were placed immediately on ice and were transferred to the laboratory. Anticoagulated blood was analyzed shortly after collection for measurements of red blood cells (RBC), hemoglobin (Hb), hematocrit (packed cell volume; PCV), and total leukocyte count (white blood cells; WBC) by microhematocrit, cyanmethaemoglobin, and standard manual methods, respectively. Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films [9].

The amounts of total serum Ca, P, Cu, Zn, and Fe; blood urea nitrogen; glucose; triglyceride; cholesterol; and HDL were measured by commercial kits (Pars Azmoon, Tehran, Iran) using an autoanalyzer (Biotecnica, Targa 3000, Rome, Italy). Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy.

Serum Ca, P, Zn, Fe, and Cu concentrations and lipid profile and urea nitrogen contents which were determined in different days were analyzed as repeated measures with the MIXED procedure of SAS [10] in a completely randomized design. Duncan's multiple range tests were used for comparison of means, considering $P \leq 0.05$ as the significant level.

## Results

Initial BW, final BW, average daily gain (ADG), DMI, and feed conversion ratio (FCR) are shown in Table 1. ADG increased ($P<0.05$) with Cu supplementation and were higher for the lambs fed diet supplemented with CuP. Feed required per unit of weight gain was significantly ($P<0.05$) lower in CuP group as compared to control and CuS groups.

The data pertaining to blood mineral profile of lambs on various Cu-containing diets are detailed in Table 2. The concentrations of blood Ca, P, and Zn were not affected by source of Cu in diet. However, Fe concentration was lower ($P<0.01$) in the Cu-supplemented groups as compared to the lambs in the control group with no significant difference among Cu-fed treatments. The Cu serum concentration for CuP (91.3 μg/dl) and CuS (87.6 μg/dl) groups were greater than the control (76.9 μg/dl) group ($P<0.05$).

The results of hematological parameters are shown in Table 3. The values of hematological parameters were within the normal range for sheep. Experimental treatment had no significant effects on the cell blood count parameters.

The serum glucose concentration was not affected ($P>0.05$) by treatments (Table 4). However, the blood urea nitrogen concentrations were significantly affected ($P<0.05$) by added Cu and was lower for CuP group as compared to the lambs in the CuS and control groups.

The serum triglyceride and HDL concentrations were not affected ($P>0.05$) by treatments (Table 4). However, the

**Table 1** Effect of copper sources on performance of lambs in different groups

| Measurement | Treatment | | | SEM | *P* value |
|---|---|---|---|---|---|
| | Control | Cus | CuP | | |
| Initial body weight (kg) | 18.2 | 17.6 | 18.1 | 0.9 | 0.99 |
| Final body weight (kg) | 27.9 | 28.1 | 29.1 | 1.1 | 0.61 |
| Average daily gain (g/day) | $150.1^{b}$ | $162.2^{a}$ | $169.3^{a}$ | 6.7 | 0.05 |
| Average dry matter intake (g/day) | 1,155 | 1,134 | 1,047 | 40.1 | 0.42 |
| Feed conversion ratio(FCR) | $7.7^{a}$ | $7.0^{a}$ | $6.2^{b}$ | 0.59 | 0.03 |

Means with different superscript letters in rows are significantly different ($P<0.05$)

*C* control, *CuS* copper sulfate, *CuP* copper proteinate

**Table 2** Effect of Cu sources on serum mineral concentration in lambs of different groups

| Measurement | Treatment | | | SEM | *P* value |
|---|---|---|---|---|---|
| | Control | ZnS | ZnP | | |
| Ca (mg/dl) | 9.98 | 9.98 | 9.76 | 0.29 | 0.70 |
| P (mg/dl) | 5.9 | 6.21 | 6.33 | 0.48 | 0.67 |
| Fe (μg/dl) | $174.2^{a}$ | $162.7^{b}$ | $160.7^{b}$ | 17.7 | 0.05 |
| Zn (μg/dl) | 72.1 | 69.3 | 68.3 | 1.1 | 0.06 |
| Cu (μg/dl) | $76.9^{b}$ | $87.6^{a}$ | $91.3^{a}$ | 7.2 | 0.03 |

Means with different superscript letters in rows are significantly different ($P<0.05$)

*C* control, *CuS* copper sulfate, *CuP* copper proteinate

concentrations of serum cholesterol were significantly affected ($P<0.05$) by added Cu. Lambs fed with CuP supplement had a lower ($P<0.05$) serum cholesterol concentration than the CuS and control animals.

## Discussion

Although DMI of lambs were not affected as Cu supplementation, ADG and gain efficiency improved ($P<0.05$) in the CuP group, which is similar to results reported by Solaiman et al. [11, 12] for Nubian doe goats fed 100 to 150 mg of additional Cu/d. Solaiman et al. [11] reported a 28 % improvement in mean ADG when Nubian doe goats were supplemented with Cu (in gelatin capsules) at levels of 100–150 mg/day for 23 weeks.

**Table 3** Effect of Cu sources on blood hematological parameters in experimental lambs

| Measurement | Treatment | | | SEM | *P* value |
|---|---|---|---|---|---|
| | Control | ZnS | ZnP | | |
| HCT (%) | 35.0 | 32.9 | 33.6 | 1.8 | 0.55 |
| RBC (106/μl) | 14.2 | 14.1 | 14.2 | 0.11 | 0.50 |
| Hb (g/dl) | 9.33 | 9.29 | 9.17 | 0.44 | 0.93 |
| MCV (fL) | 24.5 | 23.7 | 23.3 | 1.0 | 0.50 |
| MCH (pg) | 6.54 | 6.55 | 6.49 | 0.28 | 0.90 |
| MCHC (%) | 27.0 | 27.7 | 27.9 | 1.0 | 0.65 |
| WBC (103/μl) | 12.1 | 11.9 | 10.7 | 1.4 | 0.54 |
| Neut (%) | 55.1 | 52.0 | 51.1 | 5.3 | 0.68 |
| Lymph (%) | 43.5 | 46.8 | 47.3 | 5.6 | 0.66 |
| Mono (%) | 2.6 | 1.5 | 1.4 | 0.03 | 0.61 |

*C* control, *CuS* copper sulfate, *CuP* copper proteinate, *Hct* hematocrit, *RBC* red blood cell, *Hb* hemoglobin concentration, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *WBC* white blood cell, *Neut* neutrophil, *Lymph* lymphocyte, *Mono* monocyte

**Table 4** Effect of Cu sources on serum glucose and urea nitrogen concentration and lipid profile in lambs of different groups

| Measurement | Treatment | | | SEM | *P* value |
|---|---|---|---|---|---|
| | Control | ZnS | ZnP | | |
| Glucose (mg/dl) | 39.9 | 46.3 | 48.8 | 4.9 | 0.21 |
| Urea nitrogen (mg/dl) | $38.2^{a}$ | $44.5^{a}$ | $31.5^{b}$ | 3.04 | 0.003 |
| Triglycerides (mg/dl) | 23.2 | 24.6 | 26.2 | 1.7 | 0.08 |
| Cholesterol (mg/dl) | $68.3^{a}$ | $64.5^{ab}$ | $54.5^{b}$ | 5.9 | 0.04 |
| HDL (mg/dl) | 26.7 | 25.7 | 24.1 | 1.0 | 0.06 |

*C* control, *CuS* copper sulfate, *CuP* copper proteinate

Means with different superscript letters in rows are significantly different ($P<0.05$)

Conflicting results have been reported evaluating the effect of dietary Cu on performance of cattle. Gengelbach et al. [13] observed increased feed efficiency in Angus steers when 10 mg of Cu/kg of DM was supplemented to a corn silage diet containing 5.3 mg of Cu/kg of DM. Our results is in contrast to previous researches from Engle and Spears [6] in which Cu supplementation to growing and finishing steers at 20 or 40 mg Cu/kg DM from different Cu sources (organic and inorganic) reduced ($P<0.05$) ADG and feed efficiency. Several factors, such as initial Cu status of the animals, Cu content of basal diet, and concentration of Cu antagonists (Fe, S, and Mo), may affect responses of cattle to supplemental Cu [12, 14].

As expected, Cu supplementation increased the serum Cu concentration, indicating that dietary Cu levels were reflected in blood Cu concentrations. These results are consistent with Zhang et al. [15] and Dezfoulian et al. [16], who reported an increase in plasma Cu of goats and lambs which received different sources of Cu. There have been similar reports in steers [6] and calves [17]. Our results contradict to Eckert et al. [18] who reported no changes in plasma Cu of ewes receiving 10, 20, and 30 ppm copper treatments (from sulfate and proteinate). These results may point to sex and age differences in Cu metabolism.

The decrease in serum Fe concentrations as a result of Cu supplementation during the course of the study is consistent with Dezfoulian et al. [16], who reported a decrease in blood Fe of male lambs which received different sources of Cu. Du et al. [19] suggested that organic Cu complexes are absorbed via a mechanism different from inorganic Cu absorption and that it does not interfere with Fe absorption. However, this was not the case in our study for Fe. It seems that Cu supplementation above NRC [20] requirements ultimately affected Fe absorption or metabolism which is consistent with Mohri et al. [21], who reported a significant negative correlation between serum copper and iron levels in sheep.

However, Cu supplementation caused to decrease serum concentration of Fe, but hematology indicators (Table 3) suggested that no lamb displayed signs of hemolytic anemia, one of the adverse conditions caused by Cu toxicity. In agreement with our results, Heidarppor Bami et al. [22] and Naseri et al. [23] revealed over supplementation of copper in dairy calves did not affect RBC indices and different types of leukocytes. In goats, over supplementation with copper sulfate resulted in nonsignificant difference in PCV levels. WBC counts were not also affected when goats were supplemented with the amounts of 50, 150, and 300 mg/day/head of copper [11]. In another study in goats, supplementation with the amounts of 100 and 200 mg/day/head of copper had no effect on the RBC parameters and mean corpuscular volume (MCV). In copper, depleted heifers copper supplementation as sulfate or lysine at a rate of 8 or 16 mg/kg revealed no difference between groups for hemoglobin concentrations [24]. With attention to the results of previous studies and present study, it seems that low-level supplementation of copper could be safe for fattening lambs.

This study is the first to investigate the effects of dietary Cu on blood urea nitrogen concentration in male lambs. Limited research has been done on the effects of dietary Cu on protein efficiency in sheep and goats. Our results are consistent with Dezfoulian et al. [16], who reported that Cu supplementation improved CP digestibility, with proteinate treatments being more effective. In the present study, lower concentration of blood urea nitrogen in proteinate treatments suggests positive effects of Cu proteinate on rumen microorganisms rather than the host. This characteristic of Cu supplementation has been pointed out by previous studies in goats [14, 15]. Solaiman et al. [14] hypothesized that Cu supplementation might enhance rumen fermentation efficiency by decreasing the number of protozoa present in the rumen. Essig et al. [25] also reported that average protozoa count for Cu sulfate-treated steers (4.4 g/100 kg BW) was noticeably less than that in control steers 2 h after feeding. Decreasing the number of protozoa present in the rumen may be the cause to lower NH3-N concentration and lower absorption of NH3-N through the ruminal wall and thus lower BUN concentration [26].

In another hand, Reddy and Mahadevan [27] reported that Cu supplementation (daily intake of 43 to 62 mg) significantly reduced digestibility of CP in lactating cows. Differences observed between these studies for nitrogen efficiency might be caused by environmental, genetic, and dietary factors including Cu concentration in the basal diet and level and duration of Cu supplementation [6]. Interactions of Cu with other elements such as Mo, S, and Zn may also be implicated [28].

This study is the first to investigate the effects of dietary Cu on serum lipid profile in male fattening lambs. Serum cholesterol concentrations were lower ($P<0.05$) in lambs supplemented with CuP compared to CuS and control. This is in agreement with findings in cattle by Engle et al. [5]. These results are also consistent with findings in broilers when the addition of much higher concentrations of Cu (125 to 250 mg Cu/kg DM) reduced plasma cholesterol [4].

The elevated serum Cu concentrations in CuP lambs may explain, in part, the decreased serum cholesterol concentrations observed [5]. The mechanism that binds and stores Cu in the liver requires the reduced form of glutathione [29]. A decrease in the cellular concentration of the reduced form of glutathione has been shown to decrease the activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase [30–32], which is the rate-limiting enzyme in cholesterol synthesis. If supplemental Cu decreases the cellular concentration of the reduced form of glutathione, then the activity of HMG-CoA reductase could potentially be reduced, thereby decreasing cholesterol synthesis [33, 34].

The primary site of endogenous cholesterol synthesis in most mammals is the liver [35]. However, in ruminants, the primary site of cholesterol synthesis is the small intestine and adipose tissue, with the liver producing a small proportion of the total endogenous cholesterol [36]. The absorption of Cu from the intestinal lumen into the enterocyte is thought to require similar mechanisms as described for the liver. Therefore, it is hypothesized that supplemental Cu, especially organic source with high bioavailability, may decrease cholesterol synthesis in the intestine, resulting in an overall reduction in serum cholesterol in ruminants [5].

Supplemental Cu did not affect serum triglycerides (Table 3). Our results agree with the findings of Engle et al. [5] and Lee et al. [37], in which triglycerides were unaffected by Cu supplementation in steers; however, in the findings of Bakalli et al. [34], plasma triglycerides were decreased in broilers fed 250 mg of Cu/kg of DM. Findings on the effects of Cu supplementation on triglycerides are inconsistent. Animal species, breed, individual animal variability, number of animals tested, diet fed, level of supplemental Cu, or concentration of other minerals such as Zn, Mo, or S in the diet may be contributing factors [12].

The results of this study indicated that Cu supplementation from organic source increased copper status and nitrogen efficiency of lambs. Copper supplementation at levels as low as 10 mg of organic copper/kg DM can alter cholesterol concentrations in fattening lambs fed high-concentrate diets containing 8.2 mg of copper/kg DM. Further research is needed to determine the role of Cu on nitrogen and lipid metabolism, blood concentration of non-esterified fatty acids (NEFA), and β-hydroxy butyric acid (BHBA) in lambs.

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